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Modulating Reactive Astrocytes after Spinal Cord Injury using Emerging Gene Therapy with Transcription Factors

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Abstract

Spinal cord injury (SCI) leads to severe and often permanent loss of motor and sensory function due to primary tissue damage followed by secondary processes such as inflammation, oxidative stress, and apoptosis. A major barrier to repair is the glial scar, where reactive astrocytes and chondroitin sulfate proteoglycans (CSPGs) restrict axon regrowth. Rather than removing the scar, new strategies aim to reprogramming reactive astrocytes into functional neurons using transcription factors. This review highlights two recent approaches: NeuroD1-mediated reprogramming, which reduced scar-forming astrocytes, generated mature neurons, and improved locomotor recovery in mouse models; and Ngn2-mediated reprogramming, which converted astrocytes into excitatory glutamatergic neurons with demonstrated functional activity. Together, these findings suggest that astrocyte-to-neuron conversion represents a promising, cell-intrinsic approach to spinal cord repair. Although challenges remain, such as ensuring stability, specificity, and long-term

integration, emerging gene therapy with transcription factors may open new possibilities for SCI recovery.

Keywords: spinal cord injury, reactive astrocytes, Glial scar, transcription factors, neural regeneration

Introduction

Spinal Cord Injury (SCI) is a condition which results from trauma to the spinal cord which is often caused by motor vehicle accidents, falls, sports injuries, or acts of violence, resulting in motor impairments, sensory dysfunction, and disruption in the body's communication pathways due to the loss of neurons and failed axon regeneration (Ahuja et al., 2017). Its immediate effects include axon and neuronal damage, which is followed by secondary injury mechanisms such as inflammation, oxidative stress, and apoptosis, which will be covered later on in this review. Together, these processes create a highly inhibitory environment that limits the ability of the central nervous system (CNS) to repair itself after injury.

One of the main barriers to recovery after SCI is the formation of the glial scar, which, while initially protective, ultimately prevents axon regeneration and functional recovery.

Recent research has begun to explore alternative regenerative strategies that focus on modifying the injury environment rather than removing it entirely. One such approach is in vivo reprogramming, which involves converting reactive astrocytes within the glial scar into functional neurons using transcription factors such as NeuroD1 and Neurogenin-2 (Ngn2).

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This review argues that in vivo astrocyte-to-neuron reprogramming is a promising strategy for spinal cord repair, as it both reduces inhibitory scar components and promotes neuronal regeneration. By examining the mechanisms of SCI, the role of the glial scar, and recent studies involving NeuroD1- and Ngn2-mediated reprogramming, this paper evaluates the potential of this approach to improve functional recovery.

Primary Injury

The immediate effect after a SCI is physical disruption of neurons and axons at the site of impact. This damage results from the forces involved in the trauma, which can shear, stretch, or compress the neural tissues. Neurons are often damaged first, leading to disruption in neuronal networks. An important part of a neuron is the axon, which is responsible for transmitting electrical signals. When the axons are damaged, it leads to the loss of communication between the brain and areas below the site of injury (Ahuja et al., 2017). The magnitude of the initial damage determines the severity of motor and sensory dysfunction. Not only does this primary injury halt transmission, but it creates a vulnerable environment which sets a place for secondary complications, such as inflammation, oxidative stress, and apoptosis. This makes the immediate axonal and neuronal loss one of the most critical contributors to long-term outcomes (O'Shea et al., 2017).

Secondary Injury

One of the earliest and most significant secondary responses to this damage is inflammation, which quickly intensifies the injury and contributes to further neuronal loss. After a SCI, an inflammatory response is triggered, bringing immune cells like microglia and macrophages to the injury site (Silver & Miller, 2004; Burda & Sofroniew, 2014). While this helps to clear debris, it worsens the damage by releasing toxic molecules that harm nearby

neurons and support cells. This process, called immune cell infiltration, happens when immune cells migrate into a tissue and interact with the environment there. This later contributes to the formation of a glial scar, which will be covered in depth later on.

Another major contributor to secondary injury is oxidative stress. This occurs when an injury causes an overload of reactive oxygen species (ROS), leading to damage in proteins, lipids, and DNA in surrounding cells. This further weakens the spinal cord tissue and makes it harder for regeneration to occur (Ang et al., 2021). Oxidative stress can result in apoptosis, which is a form of programmed cell death. This is another secondary injury mechanism which occurs in neurons and support cells after the initial trauma. It expands the lesion over time, worsening the functional outcomes and is triggered by stress signals such as inflammation and oxidative stress (O'Shea et al., 2017). Apoptosis kills off the cells that could have survived otherwise and even supported the healing process, leading to further loss of neurons and support cells, which expands the injury site and worsens long-term recovery outcomes (O'Shea et al., 2017). Together, these secondary processes set the stage for a more permanent structural change known as the glial scar.

Glial Scar Formation and the Role of Reactive Astrocytes

As secondary injuries continue, astrocytes start to become reactive and they start to migrate towards the lesion site. This leads to the formation of a glial scar (Burda & Sofroniew, 2014). This scar is composed of cellular and molecular components, including reactive astrocytes, chondroitin sulfate proteoglycans (CSPGs), and other proteins which create a physical and chemical barrier around the injury. Initially, the glial scar plays a protective role where it seals off the injury to limit the inflammation and protect surrounding

neural tissue (Bradbury & Burnside, 2019). Over time, this transitions into a barrier, preventing regeneration. As CSPGs and other inhibitory molecules suppress axon regrowth and synaptic reconnection, it becomes problematic in the CNS where regeneration is already limited (Silver & Miller, 2004).

As mentioned before, the glial scar initially plays a protective role, but later on becomes an obstacle to regeneration due to its molecular composition. One of the main components responsible for this inhibition is CSPGs, which are large and complex molecules made of a core protein and chondroitin sulfate sugar chains. Following a SCI, these molecules are greatly upregulated in the reactive astrocytes and become densely concentrated with the scar tissue. The presence of CSPGs creates a hostile environment by actively inhibiting axon growth. When these molecules interact with the neuronal receptors and activate signaling pathways that prevent axon extension, it makes them one of the primary barriers to recovery in the central nervous system (CNS) (Silver & Miller, 2004; Bradbury & Burnside, 2019). Due to this, targeting CSPGs and their effects has become a main focus in the strategies which aim to promote regeneration.

The CNS has a very limited ability to regenerate after injury due to both internal and external factors. Internally, adult CNS neurons express low levels of growth-associated genes, which are essential for initiating axon regrowth (O'Shea et al., 2017). Externally, the injury environment becomes highly inhibitory: the formation of a glial scar releases molecules like CSPGs that block axon growth, and debris from damaged myelin releases inhibitory proteins that further prevent regeneration (Schwab & Strittmatter, 2014). Additionally, the slow clearance of this myelin debris prolongs exposure to these inhibitory

factors. Together, these internal and external barriers create a hostile environment that severely limits CNS regeneration.

In Vivo Reprogramming of Astrocytes

Due to the nature of the glial scar, astrocytes have become one of the promising targets for regenerative therapy instead of removing the entire scar, which increases risk of further destabilization at the injury site. Recent research has explored the idea of modulating astrocytes, specifically converting them into functional neurons within the scar. This approach uses in vivo reprogramming to convert reactive astrocytes into neurons by using transcription factors such as Neurogenin-2 (Ngn2) and Neuronal Differentiation 1 (NeuroD1). These are key regulators of neuronal differentiation during development, making them strong candidates for reprogramming reactive astrocytes cells into functional neurons. These transcription factors can override the astrocyte's original identity and push it toward becoming a neuron instead (Puls et al., 2020; Talifu et al., 2024). By doing this, not only are the scar-forming astrocytes reduced, but new neurons are introduced at the injury site, which could help restore lost connections and support recovery.

A key part of this approach is the use of transcription factors like Ngn2 and NeuroD1, which are normally active during brain development and help guide stem cells to become neurons. In the context of SCI, these factors are delivered using adeno-associated viruses (AAVs), which specifically target reactive astrocytes within the glial scar (Puls et al., 2020). Once inside the cells, NeuroD1 or Ngn2 can override the astrocyte's original identity and reprogram it into a neuron instead. This is important because it reduces the number of scar-forming astrocytes while introducing new neurons into the injured area. These reprogrammed neurons have been shown to express neuronal markers, make synaptic connections, and even

improve recovery in some cases (Talifu et al., 2024). To convert astrocytes into neurons in vivo, researchers need a safe and efficient way to deliver genes like NeuroD1 and Ngn2 directly into specific cells at the injury site. This is where AAVs are commonly used. AAVs are small, non-pathogenic viruses that can be engineered to carry a chosen gene, such as NeuroD1 and Ngn2, into target cells. What makes them especially useful is their ability to be combined with cell-type-specific promoters. In the context of SCI, AAVs can be designed to deliver reprogramming factors specifically to reactive astrocytes, reducing off-target effects and avoiding damage to surrounding neurons or support cells (Puls et al., 2020). This targeted delivery is essential for ensuring that only the scar-forming astrocytes are affected, making AAVs a key tool in reprogramming strategies.

NeuroD1 Based Reprogramming for SCI (Talifu et al. 2024)

Talifu et al. (2024) explored an exciting approach called in vivo reprogramming, which means directly converting one type of cell into another inside a living organism. They focused on turning reactive astrocytes, which are astrocytes activated by injury and involved in scar formation, into neurons after SCI. While previous studies tested this in brain injury models, Talifu and colleagues took this one step further by applying it specifically to the spinal cord, where regeneration is especially difficult because of the harsh environment created after injury.

To get NeuroD1, which is a key gene that encourages neuron formation into the right cells, the researchers used AAVs designed to target reactive astrocytes inside the glial scar. They did this by using the GFAP promoter, a genetic “switch” that makes sure NeuroD1 is only turned on in astrocytes. They also included a Cre-LoxP system, a genetic tool that

helped them track which cells actually became neurons, confirming that the new neurons really came from astrocytes (Talifu et al., 2024).

After delivering NeuroD1 with the AAVs, the team saw fewer GFAP-positive astrocytes, which are astrocytes marked by the GFAP protein, and more cells showing neuronal markers like NeuN and MAP2, which are proteins typical of mature neurons. This showed that the astrocytes had successfully transformed into neurons. Adding on, these new neurons had mature shapes and formed synaptic connections, which are the special junctions where neurons communicate, suggesting they were integrating into the spinal cord's existing neural circuits. The presence of synaptic structures is an important sign that these cells were not just neuron-like in appearance, but potentially functional. Talifu et al. confirmed this by showing that the reprogrammed cells expressed neuronal markers like NeuN and MAP2, proteins typically found in mature, active neurons. This indicates that the new cells were not only structurally similar to neurons but were developing the necessary features to participate in signal transmission.

Even more importantly, the study found signs of functional improvement. Mice treated with AAV-NeuroD1 showed better movement and coordination compared to untreated controls. This was measured using the Basso Mouse Scale (BMS), a widely used behavioral test that scores locomotion based on criteria like weight support, limb movement, and stepping patterns. There is a scoring system from 0 to 9 that is used to track how well mice recover movement after a SCI. A score of 0 means the mouse is completely paralyzed, and a score of 9 means it can walk normally. The scale checks for things such as if the mouse can move its joints, support its weight, coordinate both sides of the body, take steps, and keep its trunk and tail stable (Basso et al., 2006).

Although the recovery was not complete, the treated mice scored significantly higher than untreated ones. The treated mice showed progress over time, and some of them reached a BMS scale of 3-4. This means they were able to move their legs and take some weight supported steps, although the steps weren't always coordinated. It isn't full recovery, but it suggests that the new neurons may have helped rebuild some of the communication pathways disrupted by the injury. These results provide early evidence that in vivo reprogramming can do more than just change cell identity, it may actually improve spinal cord function and contribute to recovery.

While these findings are promising, Talifu et al. (2024) also pointed out that many questions remain. For example, it's still unclear whether the new neurons are long-lasting or stable over time, and how well they integrate into more complex motor circuits. However, the fact that such a transformation could be achieved using cells already present at the injury site without the need for transplantation highlights the potential of this strategy as a less invasive and more targeted treatment for SCI.

All in all, this study highlights in vivo reprogramming as a promising strategy for SCI. Since it uses astrocytes already at the injury site, it takes advantage of cells that are naturally present in the damaged area. This local approach reduces the need to introduce foreign cells into the spinal cord. It also avoids issues like immune rejection that can happen with stem cell transplants. Still, the authors note that more work is needed to see how stable and functional these reprogrammed neurons are over the long term, and to improve the gene delivery methods for future clinical use (Talifu et al., 2024).

Ngn2 Mediated Reprogramming of Astrocytes (Puls et al., 2020)

While Talifu et al. focused on NeuroD1, other research has looked at transcription factors like Ngn2, which also help promote neuron formation, for converting astrocytes into neurons (Puls et al., 2020). Different factors may produce different types of neurons or affect how quickly reprogramming happens, but NeuroD1 seems especially promising in the tough environment of the injured spinal cord.

While NeuroD1 shows a strong potential in environments such as the injured spinal cord, Puls et al. (2020) explored a different angle by using Ngn2 to reprogram astrocytes into functional neurons. In this study, the researchers demonstrate that Ngn2 can effectively convert astrocytes into excitatory glutamatergic neurons in vivo. Excitatory glutamatergic neurons in vivo are neurons created inside a living animal and are the kind that use glutamate, a neurotransmitter, to activate other neurons. Unlike NeuroD1 which generates a broader range of neuronal subtypes, Ngn2 promotes more specific neuronal identity. This is important since the type of neuron produced can influence how well it integrates into the existing neural circuit and what role it may play.

Similar to Talifu et al., Puls et al. (2020) used AAV vectors to deliver Ngn2, and they also used promoters unique to astrocytes to guarantee focused gene expression. This work was particularly intriguing since the reprogrammed neurons were electrophysiologically active, which means they could fire action potentials and take part in signal transmission, in addition to expressing mature neuronal markers. This provided stronger evidence that these were not just neuron-like cells, but truly functional neurons.

After introducing Ngn2 into the reactive astrocytes, Puls et al. observed a clear reduction in glial markers such as GFAP and a large increase in neuronal markers like NeuN

and MAP2, which confirmed a successful conversion of the astrocytes. The newly converted neurons displayed glutamatergic properties, indicating that they were excitatory and had functional characteristics with spinal cord neurons. In addition to expressing the appropriate molecular markers, these cells were able to establish synapses and produce action potentials, indicating that they may eventually be incorporated into local circuits. Although the study did not focus heavily on behavioral outcomes, it laid important groundwork by showing that Ngn2-mediated reprogramming can produce specific and functional neurons in the injured CNS (Puls et al., 2020).

Comparing NeuroD1 and Ngn2 Reprogramming Approaches

The idea of turning reactive astrocytes into neurons has been explored by both Talifu et al. (2024) and Puls et al. (2020), though they approached it with different transcription factors, NeuroD1 and Ngn2, and looked for slightly different outcomes. Talifu's group tested NeuroD1 in the especially tough environment of the injured spinal cord and found that it could generate a broader mix of new neurons. One of their biggest strengths was showing not just cellular conversion but also functional improvement, using the BMS scale to track better movement. This hinted that the reprogrammed neurons were actually helping spinal circuits reconnect.

Puls et al. (2020), on the other hand, focused more on what the reprogrammed neurons could actually do. With Ngn2, they produced excitatory glutamatergic neurons that showed real electrical activity. These cells fired action potentials, formed synapses, and expressed mature neuronal markers, all signs of being "true" working neurons. While Puls's study didn't test motor recovery the way Talifu's did, it gave strong evidence that Ngn2 can

guide astrocytes into becoming precise, functional neuron types. Taken together, the two studies suggest that NeuroD1 may be better at broad repair, while Ngn2 offers more control over the kind of neurons being created.

Conclusion

Spinal cord injury is still one of the hardest problems in medicine because of both the damage from the initial trauma and the chain reaction of secondary injury and scarring that follows. But instead of fighting against the scar, new research suggests that the cells inside it, astrocytes, might actually be part of the solution. By reprogramming reactive astrocytes into neurons with transcription factors like NeuroD1 and Ngn2, scientists have shown that repair is possible on both a structural and functional level. NeuroD1 seems especially good at driving broad neuron replacement and even improving movement, while Ngn2 produces more specific neuron types with strong functional properties. Blending these approaches, along with improving viral delivery methods and testing long-term integration, could completely change how we think about spinal cord repair. It's still experimental, but astrocyte-to-neuron reprogramming is quickly becoming one of the most promising new directions in regenerative neuroscience.

References

Ahuja, C. S., Wilson, J. R., Nori, S., Kotter, M. R. N., Druschel, C., Curt, A., & Fehlings, M. G. (2017). Traumatic spinal cord injury. Nature Reviews Disease Primers, 3(1). https://doi.org/10.1038/nrdp.2017.18

Ang, M., Gatinel, D., Reinstein, D. Z., Mertens, E., Barrio, J. L. A. del, & Alió, J. L. (2021). Refractive surgery beyond 2020. Eye, 35(2), 362–382. https://doi.org/10.1038/s41433-020-1096-5

Basso, D. M., Fisher, L. C., Anderson, A. J., Jakeman, L. B., Mctigue, D. M., & Popovich, P. G. (2006). Basso Mouse Scale for Locomotion Detects Differences in Recovery after Spinal Cord Injury in Five Common Mouse Strains. Journal of Neurotrauma, 23(5), 635–659. https://doi.org/10.1089/neu.2006.23.635

Bradbury, E. J., & Burnside, E. R. (2019). Moving beyond the glial scar for spinal cord repair. Nature Communications, 10(1). https://doi.org/10.1038/s41467-019-11707-7

Burda, Joshua E., & Sofroniew, Michael V. (2014). Reactive Gliosis and the Multicellular Response to CNS Damage and Disease. Neuron, 81(2), 229–248. https://doi.org/10.1016/j.neuron.2013.12.034

O'Shea, T. M., Burda, J. E., & Sofroniew, M. V. (2017). Cell biology of spinal cord injury and repair. Journal of Clinical Investigation, 127(9), 3259–3270. https://doi.org/10.1172/jci90608

Puls, B., Ding, Y., Zhang, F., Pan, M., Lei, Z., Pei, Z., Jiang, M., Bai, Y., Forsyth, C., Metzger, M., Rana, T., Zhang, L., Ding, X., Keefe, M., Cai, A., Redilla, A., Lai, M., He, K., Li, H., & Chen, G. (2020). Regeneration of Functional Neurons After Spinal Cord Injury via in situ NeuroD1-Mediated Astrocyte-to-Neuron Conversion. Frontiers in Cell and Developmental Biology, 8. https://doi.org/10.3389/fcell.2020.591883

Schwab, M. E., & Strittmatter, S. M. (2014). Nogo limits neural plasticity and recovery from injury. Current Opinion in Neurobiology, 27, 53–60. https://doi.org/10.1016/j.conb.2014.02.011

Silver, J., & Miller, J. H. (2004). Regeneration beyond the Glial Scar. Nature Reviews Neuroscience, 5(2), 146–156. https://doi.org/10.1038/nrn1326

Talifu, Zhang, C., Xu, X., Pan, Y., Ke, H., Li, Z., Liu, W., Du, H., Wang, X., Gao, F., Yang, D., Jing, Y., Yu, Y., Du, L., & Li, J. (2024). Neuronal repair after spinal cord injury by in vivo astrocyte reprogramming mediated by the overexpression of NeuroD1 and Neurogenin-2. Biological Research, 57(1). https://doi.org/10.1186/s40659-024-00534-w